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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

This project concerns the detection of growth inhibition of African trypanosomes and of pathogenic trichomonads by phyto-extracts from West Africa. During the period 05/01/00–04/30/01, 39 extracts received from the Walter Reed Army Institute for Research (WRAIR) and six received from the University of Dschang, Cameroon were screened against one *Trypanosoma brucei* strain and two *Trypanosoma rhodesiense* strains. Eighteen of the WRAIR and three of the Cameroon samples had IC_{50} values of ≤ 0.1 to $< 20\mu g/ml$, and were of interest for further purification and or *in vivo* studies. All 18 of the WRAIR extracts were tested in the *T. brucei* mouse laboratory model infection in standard dose response regimens of 1 to 25 mg/kg/day i.p. for 3 days. None of the extracts prolonged the lifespan of the mice as compared to infected, untreated controls.

Eight extracts were tested vs. metronidazole-sensitive and -resistant isolates of *Trichomonas vaginalis* and 18 were tested against the veterinary parasite *Tritrichomonas foetus*. Of those tested vs. *T. vaginalis*, three had MIC values of 0.3–0.6mg/ml and two extracts tested vs. *T. foetus* had MIC values of 0.3–0.6 mg/ml. These studies are continuing, with increased emphasis on *in vivo* testing of more highly purified plant extracts in an effort to determine the active agent(s) in these extracts.

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(5) Introduction

The Pace University portion of Associated Project #3 deals with activity of extracts against African trypanosomes (*Trypanosoma brucei* sub group) and human and veterinary trichomonads (*Trichomonas vaginalis* and *Tritrichomonas foetus*). African sleeping sickness is increasing dramatically up to an estimated 500,000 new cases this year (WHO, 2001), while human trichomoniasis continues to be a major STD in the United States as well as worldwide. Drug resistance to both groups of pathogens is increasing, while no new drugs have been developed for routine use. Melarsoprol and pentamidine resistance is significant for African trypanosomes, and the availability of these agents depends on the whim of the pharmaceutical firms producing them. Metronidazole has been the standard therapy for human trichomoniasis since the mid-1950's and there is no routine treatment for veterinary trichomoniasis. Thus it is important that new avenues of research be explored for drug development.

(6) Body

a) African trypanosomes. *In vitro* screens with bloodstream form trypanosomes are set up in 24 well plates using duplicate wells of 4 extract concentrations (in HMI medium) each + full-growth controls, as detailed in Bacchi et al (1996). Initial wide concentration curves were followed by narrow-ranging curves to determine IC_{50} values. Strains of trypanosomes used were: *Trypanosoma brucei*, Lab 110 EATRO (veterinary parasite); *Trypanosoma rhodesiense* KETRI 243 (human isolate), *T. rhodesiense* 243 As 10-3 (clone of KETRI 243 highly resistant to melarsoprol and pentamidine).

b) Trichomonads. The method used was the minimal inhibitory concentration (MIC) assay developed by Meingassner et al (1978). Strains used were *T. vaginalis* C1-NIH (ATTC 30001) and a metronidazole-resistant strain, CDC-085 (ATCC 50143). These are aerobically in 96 well plates with triplicate serial dilutions, and checked at 24 and 48 h.

Results

a) Trypanosomes. A total of 39 plant extracts from WRAIR and an additional 6 received from the laboratory of the late Professor Johnson F. Ayafor (University of Dschang; AP2 Phytochemistry) were screened against three strains of African trypanosomes. Another eight extracts from WRAIR were screened against *Trichomonas vaginalis* isolates and 18 were tested against the veterinary parasite *Tritrichomonas foetus*.

Of the 39 extracts received from Dr. Chris Okunji, all were tested vs. *T. brucei* Lab 110 EATRO and *T. rhodesiense* KETRI 243, and 38 were tested vs. the drug-resistant clone, KETRI 243 As 10-4 (Table 1).

The following extracts had sufficient activity ($IC_{50} \leq 20$ μg/ml) against one or more isolates to warrant further consideration for *in vivo* testing or further purification: SU#: 1863, 1866, 1869, 1870-1976, 1878-1881, 1886, 1889, and 1891. These samples were obtained from the following plants: *Aspilia africana, Chamaecrista mimosoides, Combretum dulchipetalum, Cryptolepis sanguinolenta, Enantia chlorontha, Hoslundia opposita, Icacina trichanta, Phyllanthus amerus, Pleiocarpa pycnantha, Trimfetta tomentosa,* and *Uvaria chamae*. Those extracts having activity at < 5 μg/ml were: SU#: 1872, 1874, 1878, 1880, 1891. We regarded these as prime candidates for *in vivo* trial and have done preliminary *in vivo* tests with these extracts.

Before Professor Johnson Ayafor died in November, he had provided us with a large number of extracts. Twenty-six of these were screened against the three isolates and reported in the previous report. We are in contact with Professor Ayafor's colleague, Dr. Apollinaire Tsopmo (Chemistry Department, University of Dschang) and received an additional nine extracts for invitro screening and have partial results with six of these. Two of the six, ASP and TZM, show high activity (0.5–2.35 μg/ml).

b) Trichomonads. A total of eight WRAIR extracts were tested against *T. vaginalis* extracts *in vitro* (Table 3). Of these SU: 1863, 1870, 1873, and 1877 had MIC values < 1 mg/ml, and are considered of interest for further study. This screen was limited because of continuing growth and contamination problems with the Trichomonas isolates. Extracts 1866–1902 (a total of 22) were screened for activity vs. Tritrichomonas foetus, a veterinary parasite for which there is no cure. Of these 1874 and 1895 had MIC values of 0.3–0.6 mg/ml, and are considered of interest for further study.

c) *In vivo* testing. A total of 18 plant extracts gave IC_{50} values of $\leq$ 20 μg/ml in the trypanosome screen. Since many of the extracts were oils which had to be solubilized in DMSO, we had on hand highly concentrated stock solutions of the extracts. Using 50% DMSO to dilute these stocks, we set up *in vivo* experiments in the *T. brucei* Lab EATRO mouse mode. Dose curves were 1, 5, 10, 25 mg/kg/day i.p. once daily for 3 days. Three mice 25–30 g were used per dose point, and three were untreated, infected controls. The extracts tested were: SU1863, 1866–1876, 1879–1881, 1886, 1889–1891. None were curative, although SU1875 and 1891, prolonged the lifespan of 10 and 25 mg/kg groups by 2–3 days beyond that of the infected, untreated controls. Doses higher than 25 mg/kg were not possible because the volume of 50% DMSO given would have needed to be increased to a toxic level. SU1889 was highly toxic at doses > 1 mg/kg.

(7) Key Research Accomplishments

- Identification of 15 WRAIR plant extracts and another 2 from Cameroon (AP #2) out of a total of 45 extracts tested which had IC_{50} values of $\leq$ 20 μg/ml for three African trypanosomes isolates.
- An additional 6 of the WRAIR and 2 of the AP #2 extracts had IC_{50} values of $\leq$ 5 μg/ml for 3 African trypanosomes isolates.
- Since the purity and the potency of these extracts is not known with respect to the active agents, we believe all of these extracts are candidates for further purification and/or *in vivo* testing.
- The most active extracts vs. *T. vaginalis* were not those most active against the African trypanosomes, indicating some degree of specificity is present, and fewer of these had MIC values in the active range 0.3–0.6 mg/ml.

(8) Reportable Outcomes. None

(9) Conclusions

Research since May 2000 has identified a number of plant extracts having significant *in vitro* growth inhibitory activity against African trypanosomes and pathogenic trichomonads. Additional supplies of the extracts are needed to effect extended (5 or 7 day) dosing regimens. Moreover, additional purification of active extracts is needed to allow better control of *in vivo* dosing.

In comparing *in vitro* activities of extracts, it is evident that a degree of specificity was again present – for example, SU-1870 and 1880 were highly effective vs. trypanosomes but not *T. vaginalis* isolates.

Economically, this is an important program for Nigeria and Cameroon: both are endemic for human and veterinary trypanosomiasis, while STD infections, including *T. vaginalis* and bovine trichomoniasis are a significant source of human suffering and an economic drain. There is an urgent need for new and inexpensive drugs for trypanosomiasis. Treatment of human trichomoniasis depends solely on metronidazole, and there is no agent currently available for bovine trichomoniasis [metronidazole kills the microbial flora of the rumen and cannot be given to cattle]. Development of anti-protozoal agents from local plants would be a major factor in the well-being of these populations and a boost to local and national economics.

(10) References

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Table 1. Activity of plant extracts vs. growth of African trypanosomes *in vitro*. Bloodforms trypanosomes were grown in 24 well culture dishes (1 ml/well) in HM1-18 medium (Hirumi & Hirumi 1989). One half of the culture volume was replaced daily with fresh medium plus drug. Each extract was dissolved in 100% DMSO and diluted with medium. Cells were counted daily with a Coulter counter. Data are as IC_{50} values in μg extract/ml culture. Four strains were used: *T. b. brucei* Lab 110 EATRO, and three *T. b. rhodesiense* clinical isolates from the Kenya Trypanosomiasis Research Institute (KETRI). All data from 48 hr cultures. Control cell counts averaged 5 x 10^6 cells/ml at 48 h. (Data through May 2001).

	IC_{50} (μg/ml)			
	EATRO 110	**KETRI 243**	**KETRI 269**	**KETRI 243 As10-3**
SU-1863	2.4	20	-	20.5
SU-1864	71	225	-	220
SU-1865	82	140	-	165
SU-1866	6.6	36	-	25
SU-1867	19.5	54.5	-	61
SU-1868	11	16	-	18.5
SU-1869	13.5	11	-	22
SU-1870	7.3	6.4	-	6.6
SU-1871	18.5	17.5	-	22.5
SU-1872	0.66	5.7	-	4.8
SU-1873	6.9	9.8	-	8.4
SU-1874	5.0	1.75	-	28.5
SU-1875	7.65	5.75	-	6.7
SU-1876	22	15.5	-	24.5
SU-1877	28.5	59	-	24.5
SU-1878	0.84	4.6	-	0.92
SU-1879	12.5	13.5	-	18.5
SU-1880	0.97	0.69	-	4.2
SU-1881	17.5	22.5	-	19.5
SU-1882	205	215	-	165
SU-1883	71	200	-	165
SU-1884	63.5	45.5	-	65

Table 1 (continued)

	IC_{50} (μg/ml)			
	EATRO 110	**KETRI 243**	**KETRI 269**	**KETRI 243 As10-3**
SU-1885	205	225	-	180
SU-1886	64	7.9	-	135
SU-1887	129	150	-	160
SU-1888	145	25	-	185
SU-1889	3.7	36	-	187.5
SU-1890	22.5	73	-	14.5
SU-1891	0.57	2.6	-	33.25
SU-1892	180	190	-	140
SU-1893	48	21.5	-	74
SU-1894	20	21.9	-	16.5
SU-1895	200	64	-	150
SU-1896	135	175	-	
SU-1897	125	295	-	330
SU-1898	28.5	28.5	-	20.1
SU-1899	94	190	-	85
SU-1902	220	225	-	160
SU-1903	78	175	-	125
Pentamidine	0.00098	0.00080	-	-
Melarsen Oxide	0.0075	0.016	-	-

Table 2. IC_{50} values for Ayafor extracts (Cameroon, AP #2). Extracts were tested vs. trypanosome isolates grown in bloodforms in HMI-18 medium containing 20% fetal bovine serum. Coulter counts were made daily and IC_{50} values determined after 48 h. as in Table 1 (May 2001).

	IC_{50} (μg/ml)			
		KETRI		
	Lab110 EATRO	**243**	**269**	**243 As 10-3**
ASP	0.5	1.5	-	-
ASS_2	28	26.5	-	-
ASS_4	30.5	16	-	51
ASS_5	55	50.1	-	-
TZM_{1A}	21.5	18	-	-
TZM_1	2.35	-	-	-
TZM_4	-	-	-	-
TZM_4 HCl	-	-	-	-
TZM_3	-	-	-	-

Table 3. Activity of plant extracts vs. growth of *Trichomonas vaginalis in vitro*. Assays were performed in 200 µl wells in triplicate. Each extract was dissolved in DMSO and diluted with growth medium. Assays are read at 24 and 48 hours and MIC value determined by the lowest concentration of extract at which triplicate wells show no growth (Meingassner et al 1978). Strain 30001 is Metronidazole sensitive, strain 50143 is Metronidazole refractory.

	MIC (mg/ml)					
	ATCC 30001		ATCC 50143		KVI	
Extract	24h	48h	24h	48h	24h	48h
SU 1863	1.3	0.6	<1.3	0.6		
SU 1864	1.3	<1.3	<1.3	<1.3		
SU 1865	<1.3	<1.3	<1.3	<1.3		
SU 1868	1.3	0.6	1.3	1.3		
SU 1870			0.3	0.3		
SU 1873			1.3	0.6		
SU 1876			<1.3	<1.3		
SU 1877			<1.3	0.6		

SU 1866					1.3	1.3
SU 1867					>1.3	>1.3
SU 1869					>1.3	1.3
SU 1872					>1.3	1.3
SU 1874					0.6	0.3
SU 1875					>1.3	1.3
SU 1878					1.3	1.3
SU 1879					1.3	0.6
SU 1880					>1.3	1.3
SU 1881					>1.3	1.3
SU 1882					1.3	1.3
SU 1883					>1.3	1.3
SU 1884					1.3	1.3
SU 1891					>1.3	1.3
SU 1892					>1.3	>1.3
SU 1893					1.3	1.3

Table 3 (continued)

	MIC (mg/ml)					
	ATCC 30001		ATCC 50143		KVI	
Extract	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
SU 1894					>1.3	>1.3
SU 1895					0.63	0.63
SU 1899					1.3	1.3
SU 1900					>1.3	>1.3
SU 1901					>1.3	>1.3
SU 1902					1.3	1.3
Metronidazole		0.003		0.40		0.003